



## Significance of Ascitic Fluid Cytology for the Detection of Ovarian Tumour: an experience in a Tertiary Care Centre.

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### Abstract

**Background:** Ascitic fluid cytology is a simple and non - invasive method for the diagnosis of various pathological conditions including ovarian malignancy. The detection of ovarian tumours by the conventional cytological smear method is quite challenging. This study aims to evaluate the accuracy of ascitic fluid cytology in the diagnosis of ovarian tumours and its histopathological correlation. **Methods:** A retrospective study was done for 4 years from January 2016 to December 2019 in a tertiary care centre in Western Odisha. The ascitic fluids with suspicion of malignancy clinically and radiologically were processed as per conventional method and histopathologically correlated. The sensitivity, specificity, negative and positive predictive values were calculated. **Results:** Out of 144 cases, 58 cases were diagnosed as benign ovarian tumours, 3 borderline and 83 cases as malignant ovarian tumours. The histopathology of these cases showed 4 false-positive cases and 32 false-negative cases. The sensitivity, specificity, positive predictive value, and negative predictive value in our study were 62.8%, 93.1%, 93.1% and 62.8% respectively. The diagnostic accuracy was calculated as 75%. **Conclusion:** Ascitic fluid examination is a highly specific and relatively sensitive test for detecting ovarian carcinoma, especially in stage 3 and 4 tumours. As patients usually present late, it helps in the staging of tumour and the prognosis of patients.

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## INTRODUCTION

Ascites is the accumulation of a large amount of fluids within the peritoneal cavity. Clinically, ascites is a consequence or complication of a number of diseases, including hepatic, cardiac, and renal diseases, infection, and malignancy.<sup>[1]</sup> Malignant ascites denotes the presence of malignant cells in the peritoneal cavity. It accounts for 10% of all cases of ascites.<sup>[2]</sup> Gynaecological and gastrointestinal malignancies are the common malignancies that present as malignant ascites.<sup>[3]</sup> About 52%-54% of patients presented with ascites at the time of initial cancer diagnosis.<sup>[3,4]</sup> The pathophysiology of malignant ascites is multifactorial and the two basic pathological mechanisms include increased vascular permeability and obstructed lymphatic drainage.<sup>[5]</sup> In the case of ovarian tumour, patients often present in advanced stages (FIGO stage III-IV) because the disease is asymptomatic in its early stage and associated with a poor prognosis.<sup>[6]</sup> It leads to poor quality of life and early mortality. The survival rate in advanced stages (III and IV) is 5-20%.<sup>[7]</sup> The malignant epithelial tumours of the ovary are by far the most frequently encountered malignant form of ovarian tumour and the most common cause of ascitic fluid.<sup>[7,8]</sup> The cytological examination of the ascitic fluid, free peritoneal fluid and peritoneal washing is a well-accepted method for the investigation of patients with epithelial ovarian cancer.<sup>[9]</sup> In 1976; FIGO incorporated it into staging protocols.

Significant survival benefits were demonstrated by the FIGO staging.

The finding of malignant cells in ascitic fluid often helps clinicians to confirm their clinical findings. It is important not only in diagnosis but also in staging and prognosis of the patients for malignancy.<sup>[7]</sup>

The objectives of this study were to determine the pattern of cancers causing malignant ascites as well as to test the validity of ascitic fluid cytology in the detection of ovarian tumours, to determine sensitivity, specificity, false positive and false negative results by confirming the diagnosis by histopathology.

## MATERIALS AND METHODS

The retrospective study was done for a period of four years from January 2016 to December 2019 in the Department of Pathology, Veer Surendra Sai Institute of Medical Sciences and Research, Burla. Total 144 findings of ascitic fluid cytology of female patients were included who were clinically or radiologically diagnosed with ovarian mass with ascites. The patients' clinical and cytological findings were collected from cytology section of our department. About 10 ml of fresh peritoneal fluid samples were processed as per the conventional method. Around 5 ml of sample was taken in a test tube and centrifuged at 2500 rpm for 15 minutes. The supernatant fluid was then discarded and minimum of 2 thin smears were

prepared from the sediment. One smear was fixed in isopropyl alcohol for half an hour and stained with Papanicolaou stain. The other dry smear was stained by Diff - Quick stain. The smears were studied by a group of pathologists and the results were reported as positive or negative for malignancy.

The corresponding histopathological samples of ovarian mass were fixed in 10% formalin and processed by routine procedure, paraffin embedding and Haematoxylin - Eosin staining. Functional ovarian cysts were excluded from the study. Histopathologically, tumours were classified by applying WHO 2014 diagnostic criteria.<sup>[10]</sup> The results were then calculated for sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy.

## RESULTS

In our study, a total of 144 ascitic fluid samples were studied which were sent to the cytology section of our department. Table 1 shows the age-wise distribution of ovarian tumours which were presented with ascites. The most common age group for benign ovarian tumour was 51-60 years, followed by 31-40 years. The age group

61-70 years had the fewest patients. Malignant tumours were more common in the fifth and sixth decade.

Among 144 cases of ovarian tumours, 40.3% were benign, 2.1% borderline, and 57.6% were malignant. The histopathological distribution and ascetic fluid cytology findings in various benign ovarian tumours are presented in Tables 2. The most common benign lesions were surface epithelial tumours 49 cases(84.5%), followed by sex cord- stromal tumours 5 cases (8.6%), and germ cell tumours 4 cases (6.9%). Of the 58 benign tumours, 33 (56.9%) were serous cystadenoma, 16 (27.6%) mucinous cystadenoma, 4 cases (6.9%) each of dermoid cysts/mature cystic teratomas and fibrothecoma and 1 case of fibroma (1.7%).

All these cases were negative for malignant cells on ascitic fluid cytological examination except 4 false-positive cases (3 cases of serous cystadenoma and 1 case of dermoid cyst) which on histopathology reported as benign tumours with no omental deposits.

**Table 1:** Age wise distribution of ovarian tumour

Age group (in years)	Type of ovarian tumours			Total
	Benign	Borderline	Malignant	
0-20	3	0	3	06
21-30	10	1	4	15
31-40	14	1	12	26
41-50	12	1	31	44



51-60	17	0	24	39
61-70	2	0	10	12
71-80	0	0	02	02
<b>Total</b>	<b>58</b>	<b>3</b>	<b>83</b>	<b>144</b>

**Table 2:** Benign ovarian tumours (total = 58, 54 true negative + 4 false-positive)

Histological types	Total cases	Number of negative cytological findings	Number of positive cytological findings	Percentage of false-positive findings
Serous cystadenoma	33	30	3	9.1
Mucinous cystadenoma	16	16	0	0
Dermoid cyst	4	3	1	25
Fibroma	1	1	0	0
Fibrothecoma	4	4	0	0
<b>Total</b>	<b>58</b>	<b>54</b>	<b>4</b>	<b>6.8</b>

**Table 3:** Borderline and malignant ovarian tumours (total 86= 54 true positive + 32 false-negative).

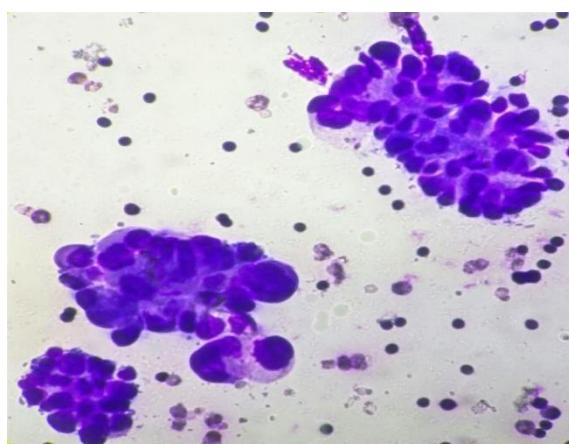
Histological types	Total number of histological type	Number of positive cytological findings	Number of negative cytological findings	Percentage of false-negative findings
Serous cystadenoma (borderline)	2	0	2	100
Serous carcinoma	60	43	17	28.3
Mucinous cystadenoma (borderline)	1	0	1	100
Mucinous carcinoma	10	7	3	30
Seromucinous carcinoma	1	0	1	100
Dysgerminoma	1	0	1	100
Mixed germ cell tumour	1	0	1	100
Yolk sac tumour	1	0	1	100
Endometrioid carcinoma	2	1	1	50

<b>Granulosa cell tumour</b>	2	0	2	100
<b>Squamous cell carcinoma in teratoma</b>	1	0	1	100
<b>Metastatic tumour (Krukenbergtumour)</b>	4	3	1	25
<b>Total</b>	86	54	32	37.2

The histopathological distribution and ascitic fluid cytology findings in various borderline and malignant ovarian tumours are presented in Tables 3.

Serous carcinomas (69.8%) were found to be the most common ovarian malignancy among borderline and malignant ovarian tumours (86 cases), followed by mucinous carcinoma (11.6%), and metastatic tumours (4.65%). We found 3 cases of borderline

ovarian tumours (2 serous and 1 mucinous), as well as 2 cases of endometrioid carcinoma and granulosatumour. 1 case each of teratoma with squamous cell carcinoma, seromucinous carcinoma, dysgerminoma, yolk sac tumour, and mixed germ cell tumour were identified histologically, which were found negative on cytology examination.



**Fig.1:** Ascitic fluid cytology of serous carcinoma showing papillary fragments comprised of atypical pleomorphic epithelial tumour cells. (Diff- Quick stain, x 400)

**Table 4:** Ascitic fluid cytology findings in correlation with histopathology.

<b>ASCITIC FLUID CYTOLOGY</b>	Positive for malignant cells = 58 cases	Negative for malignant cells = 86 cases	<b>Total</b> = 144 cases
<b>HISTOPATHOLOGY</b>	Malignant - 54	Malignant -32 (false negative)	86
	Benign - 4 (false positive)	Benign - 54	58

The following parameters were calculated as follows:

- Sensitivity = (True positive/True positive + False negative) x 100 =  $(54/54+32) \times 100 = 62.8\%$
- Specificity = (True negative/True negative + False positive) x 100 =  $(54/54+4) \times 100 = 93.1\%$
- Positive predictive value = (True positive/True positive + False positive) x 100 =  $(54/54+4) \times 100 = 93.1\%$
- Negative predictive value = (True negative/True negative + False negative) x 100 =  $(54/54+32) \times 100 = 62.8\%$
- False positivity =  $(4/144) \times 100 = 2.78\%$
- False negativity =  $(32/144) \times 100 = 22.2\%$

Diagnostic accuracy = (True positive + True negative/ total no.) x 100 =  $(54+54/144) \times 100 = 108/144 \times 100 = 75\%$

## DISCUSSION

Ovarian cancer is one of the most common gynaecologic cancers after cervical and uterine cancer.<sup>[11]</sup> However, due to the relative lack of specific signs and symptoms of this disease, lack of active screening, and early detection technique more and more women are believed to visit their doctors in the advanced stage of the disease, complicated with ascitic fluid, resulting in low overall cure rates.<sup>[8,12-14]</sup> The patients often present with ascites as a first manifestation. Cytological examination of serous effusion has increasingly gained acceptance in clinical medicine, to such an extent that a positive diagnosis often is considered

the definitive test and obviates exploratory surgery, although admittedly many other clinical confirmatory, but not conclusive, findings of malignancy may be present.<sup>[15]</sup> The positive cytological findings are important in the subclassification of stages I and II of the disease and it represents an important predictive factor in prognosis and recurrence.<sup>[16]</sup> Ovarian cancer cells mainly metastasize within the peritoneal cavity, which involves exfoliation from the primary tumor, survival, and transport in the peritoneal fluid followed by metastatic colonization of the organs within the peritoneal cavity.<sup>[17]</sup> Other routes of metastasis are lymphatic and haematogenous. <sup>[18,19]</sup>

Ovarian malignancy mainly occurs in older age group. Katsume et al reported that benign epithelial ovarian tumours may occur at any age, but most commonly during 5<sup>th</sup> decade, and malignant ones are mostly seen between the ages of 40-70 years.<sup>[20]</sup> In our study, benign tumours were mostly between the 4<sup>th</sup> to 6<sup>th</sup> decade and malignant tumours were mostly seen in the 5<sup>th</sup> and 6<sup>th</sup> decade.

We found 4 (6.8%) false-positive cases and 32 (37.2%) false-negative cases. Our findings correlated with other studies which also showed a high percentage of false-negative cases. Živadinović R et al found false-positive cases as 6.38% and false-negative as 30.02%.<sup>[16]</sup> Oscar L reported false-positives in 4.5% of cases and false-negatives in 20% of cases.<sup>[21]</sup> We



found 3 cases of serous cystadenoma and 1 case of dermoid cyst that was reported as positive for malignant cells.

The main reason for a false-positive diagnosis in various studies has been described as the misinterpretation of reactive mesothelial cells as malignant epithelial cells. Other potential pitfalls include the presence of psammoma bodies, endometriosis, and endosalpingiosis.<sup>[22,23-25]</sup> The main cause of false-positivity in our case was the misinterpretation of reactive mesothelial cells as malignant cells, which were misidentified as adenocarcinoma cells and reported as positive for malignant cytology. Reactive mesothelial cells usually present as clusters of epithelial cells with occasional cell ball or papillary cluster formation. The cells show cellular enlargement, dense cytoplasm and large nuclei with increased nuclear to cytoplasmic ratio. Occasionally the cells might be vacuolated or contain prominent nucleoli. The presence of cellular "windows" might help to identify the cells as mesothelial. Cells from fallopian tubes in pelvic washings may also lead to false-positive diagnosis.<sup>[21]</sup> The cytoplasm of neoplastic cells lacks a typical two-zone pattern (easily seen in Diff-Quick stained preparations), with eccentric nuclei touching the cell membrane without any zone along the nuclear margins due to lack of microvilli and favour adenocarcinoma (if histiocytes are excluded by studying nuclear details in Pap stained preparations).<sup>[26]</sup> The factors

responsible for the high percentage of false-negative cytological results of ascitic fluid include poor distribution of cells in the sampled ascitic fluid, infrequent cell exfoliation, and interpretive errors.<sup>[21]</sup>

In our study, the most common malignant tumour was found to be serous carcinoma. Serous carcinoma usually presents as cellular specimens containing single cells or poorly cohesive irregular cell clusters with large, pleomorphic nuclei and prominent nucleoli.<sup>[21]</sup> The papillary clusters of cancer cells with nuclear chromasia are also seen. (Fig-1) Peritoneal washings involved by endometrioid carcinomas display loose, 3-dimensional clusters of cells with eccentric, pleomorphic nuclei and abundant delicate cytoplasm, coarse chromatin pattern, and prominent nucleoli.<sup>[21]</sup> Other studies also showed serous carcinoma as the commonest malignancy.<sup>[7,16]</sup>

The sensitivity, specificity, positive predictive value and negative predictive value in our study were 62.8%, 93.1%, 93.1% and 62.8% respectively. The other studies reported sensitivity as high as 97% by Runyon et al and 94% by Cheng et al.<sup>[27,28]</sup> Karoo et al however showed somewhat lower sensitivity which was 60% and high specificity of almost 100%.<sup>[29]</sup> According to Živadinović R et al the sensitivity of peritoneal cytology was 68.92%, specificity 93.61%, positive predictive value 89.65%, and negative predictive value is 78.57%.<sup>[16]</sup>



These correlated well with the present study.

The cytological examination of ascitic fluid is important for staging and management of ovarian cancer and thus found to have prognostic significance.<sup>[30]</sup> A study by Shen-Gunther Jet al showed that ovarian malignancies in the early stages (I and II) produced ascites only in 17% of the cases, whereas in advanced stages (III and IV), 89% produced ascites.<sup>[7]</sup> 5-year survival rate for epithelial ovarian cancer is reported to be directly related to the surgical stage.<sup>[21]</sup>

The majority of epithelial cancers have an exophytic growth on the ovarian surface, allowing them to have direct contact with the peritoneal cavity. The result of secondary cytology after the treatment is also an important independent prognostic marker that is highly correlated with the optimal effect of surgical treatment, recurrence and overall survival rate. In positive secondary cytology, survival is 13 to 32 months, while in negative cytology, it is > 48 months.<sup>[31]</sup> Early detection and prompt management is the key to improve survival rate among the affected.

About two-thirds of ovarian cancer mortality is attributable to high-grade serous carcinoma.<sup>[32]</sup> The diagnostic

accuracy calculated in our study was 75%. We concluded that the peritoneal cytology of ascitic fluid is highly specific (93.1%) but less sensitive (62.8%). The lower sensitivity of cytodiagnosis of effusions was mainly due to bland morphological details of cells, overcrowding or overlapping of cells along with cell loss and changes due to improper preservation.

## CONCLUSION

The development of ascites has unfavourable outcomes and detrimental effects on the overall quality of patients. The conventional ascitic fluid cytology method is easy to perform, cost-effective and safe investigation. It is relatively sensitive and specific for the diagnosis of ovarian cancer. The diagnosis can be obtained in a very short period of time. It is very useful in hospital settings like ours, where patients come from distant villages and the clinicians need urgent reporting before opening the abdomen. In developing countries like ours this method should continue to be a first line investigation to screen out patients with suspicion of malignancies. The careful and meticulous screening of cellular details of cells helps to reduce the false positive and false negative results of ascitic fluid cytology in ovarian cancer.

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